SECRETION OF AMYLASE BY PLANT ROOTS[1]

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(WITH TWO FIGURES)

The fact that green plants are able to absorb certain organic substances by means of the roots, and to utilize these substances, suggests the question whether the roots of plants secrete enzymes in a manner comparable to various fungi, digesting in the culture medium, etc., the various organic substances that might be supplied. Various investigators have incidentally touched the subject, but the evidence obtained is conflicting and not at all conclusive.

LAURENT (3) reported the inversion of saccharose when this sugar was present in the culture media, and he ascribed this to the enzyme invertase secreted by the roots of corn or of peas. Starch was likewise transformed, but LAURENT ascribed this transformation to diastase secreted by the seed. MAZÈ (4) reported inversion of saccharose, but in 1911 (5) he stated that there was no enzyme secretion by the roots, and that starch was absorbed directly. WOHLLEBE (8), investigating the secretion of amylase by roots, came to the conclusion that there was a very weak secretion of amylase by the root hairs, and in some cases secretion of amylase was effected by the disconnected root-cap cells. The senior writer of this paper suggested in a previous publication (2) that invertase is secreted by the roots.

In view of the indefiniteness of information on the subject, it seemed advisable to investigate the problem. The first experiments were made on the secretion of amylase, and the results obtained constitute the basis for this paper.

Pfeffer's was the nutrient solution employed. It was made up according to the following formula: $Ca(NO_3)_2$ 4 gm., KNO_3 1 gm., K_2HPO_4 1 gm., $MgSO_4$ 1 gm., KCl 0.5 gm., $FeCl_3$ 100 mg., distilled water 6 l. Merck's soluble starch was used throughout the experiments.

[1] Contribution from the Laboratory of Plant Physiology, Cornell University.

The plants were grown under sterile conditions.[2] The seeds were sterilized by the calcium hypochlorite method (7). After the seeds were sterilized, they were planted in test-tubes on sterilized

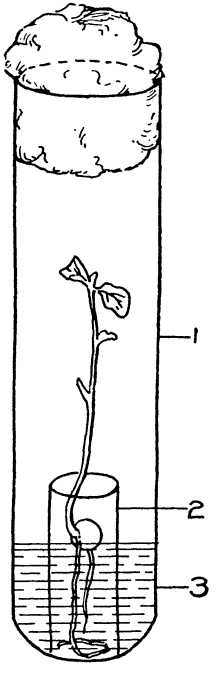

FIG. 1

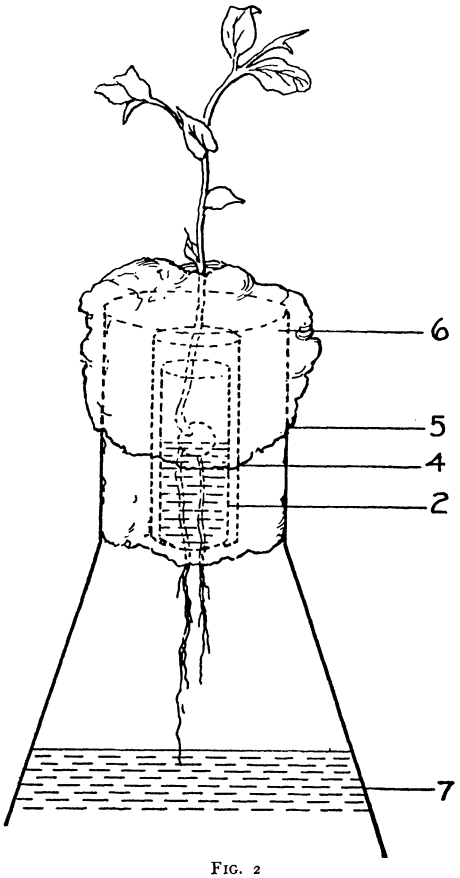

FIG. 2

FIGS. 1, 2.—*1*, sterile culture tube; *2*, germination tube; *3*, 1 per cent agar; *4*, outer tube; *5*, Erlenmeyer flask; *6*, sterile cotton; *7*, nutrient solution.

agar, as shown in fig. 1. When the seedlings were sufficiently developed, they were transferred to the culture vessels. The culture vessels used were Erlenmeyer flasks of 1 or 2 l. capacity.

[2] The writers are indebted to Dr. J. K. WILSON for the method of growing plants under sterile conditions.

The flasks were stoppered with cotton plugs, each provided with a glass tube passing through the center, this tube also being plugged with cotton. The culture flasks with solutions were sterilized in the autoclave for 30 minutes at 15 pounds pressure.

When the seedlings were of adequate size, they were transferred to the culture vessel. Transfer was made when the roots reached the bottom of the tube and were curled about, and the tops had attained a height of about 5 cm. By use of a heavy platinum needle, the tube, together with the inner core of agar and the seedling, was withdrawn from the test-tube and transferred to tube 4 of the culture vessel (fig. 2). The tube 4 was slightly drawn at the base so as to prevent tube 2 from passing through into the culture solution. Sterilized cotton was then packed about the seedling in tubes 2 and 4. The cultures when set up appeared as shown in fig. 2. After being kept for a few days in the laboratory, the cultures were transferred to the greenhouse.

The particular advantage of this form of culture, from the standpoint of studying the secretion of enzymes by the roots, is that the seeds are kept entirely out of contact with the culture solution, and any enzymes derived from the seeds are held in the agar, which with the passing of time loses its water and hardens to a flaky mass.

In spite of all the precautions taken, cultures occasionally became contaminated. All such cultures were rejected. At the conclusion of the experiment, the culture solutions were brought to the original volume and analyzed. In the first cultures, tests were made for contaminating organisms, but these and other similar experiments indicated that if the culture solutions were clear at the conclusion of the experiment there was no contamination. Consequently, in the later experiments, no platings were made of the culture solutions. Data were collected also on the weights of roots and tops. Detailed methods of procedure are described under the different experiments.

For the first experiment two cultures were set up, following the method described, using 2 l. flasks, in each of which was placed 1800 cc. of the culture solution containing 0.25 per cent of soluble starch. A variety of corn known as Leaming was employed. The

cultures were grown in a greenhouse, under favorable conditions, from November 14, 1916, to December 5, 1917. One of the cultures became contaminated, so that data were obtained from only one culture. Reducing sugar was determined by KENDALL'S method (1).

The dry weights of roots and tops were respectively 172 and 430 mg.; the total weight was 602 mg.; and the increase in weight over the original weight of the seed was 262 mg. Determinations made of the reducing sugar in 100 cc. lots of both the culture solution and the control solution gave 22.18 mg. of copper for the former, and 11.25 mg. for the latter; or an increase of 10.93 mg. of copper for a 100 cc. solution (about 6 mg. of maltose). A sample of the culture solution and one of the control, with 2 per cent of toluene added to each, were incubated for one week at 30° C., and showed no increase in reducing sugar.

In this preliminary experiment there was noted a slight increase in reducing sugar, but the increase was so small as to be without significance. Furthermore, the fact that there was no increase of reducing sugar on incubation leads to the conclusion that the enzyme amylase was not secreted into the culture solution.

The conditions of the second series of experiments were the same as for the preceding, except that liter flasks were employed as culture vessels, and 1100 cc. of the culture solution was used. A variety of white dent corn known as Boone County White was used. The cultures were grown for a period of 51 days.

At the conclusion of the experiment, the culture solutions were brought to their original volume and samples were kept for analysis. To the sample solutions was added 2 per cent of toluene, and two weeks elapsed before the solutions were analyzed for reducing sugars. Analyses were made by the Munson Walker method. Data are given in table I. The data indicate that in the culture solutions there is a slight increase in reducing sugars, but not sufficient to warrant the conclusion that there is any amylase secretion. The objection might be raised that in these cultures there can be no accumulation of reducing sugars, because they are utilized as fast as produced, which is possible; but the fact that the increase is so slight, even after two weeks of incubation, supports the theory that no amylase was present.

In the third experiment the procedure was similar to that in the preceding experiments. A white dent corn was used, and also Canada field pea. In addition to using Pfeffer's solution plus soluble starch, a number of cultures were made in which Pfeffer's solution alone was employed, to see whether any reducing sugars were secreted. Two liter flasks were used. The concentration of starch was approximately 0.35 per cent. The duration of the

TABLE I

Culture solution	Dry weight (in gm.)				Reducing sugar as CuO (in mg.)	
	Roots	Tops	Total	Gain	In 100 cc. sample	Increase per 100 cc.
Pfeffer's solution	0.6	1.05	1.65	1.3	None	None
Pfeffer's solution +0.25 per cent starch	0.5	2.1	2.6	2.3	14.5	6.4
Pfeffer's solution +0.25 per cent starch	0.5	1.7	2.2	1.9	10.0	1.9
Pfeffer's solution +0.25 per cent starch	0.7	2.15	2.85	2.55	12.9	4.8
Pfeffer's solution +0.25 per cent starch; no plant	.....	.....	.....	.....	8.1	.....

experiment was 47 days. At the conclusion of the experiment, the culture solution and the controls were made up to their original volumes, and 20 cc. portions were taken and were analyzed by Shaffer's method (6) for reducing sugars. Sample lots of each were also incubated with 2 per cent of toluene at 32° C. for 10 days, and the reducing sugars again determined. The data are given in table II.

There is a very slight increase in reducing sugars in some of the culture solutions over that in the control, but not enough to be of any significance. Furthermore, after 10 days' incubation there was no increase in the amount of reducing sugars.

Finally, to prove that the soluble starch is not utilized directly or indirectly to any appreciable extent, the following procedure was undertaken. Sample lots of the control and culture solutions were hydrolyzed and reducing sugars were determined. It was found that the control and culture solutions showed the same amount of

reducing sugar, which was equivalent to 73 mg. of glucose per 20 cc. of the culture solution.

In conclusion, therefore, it may be stated that neither *Zea mays* L. nor *Pisum arvense* L. is capable of utilizing soluble starch

TABLE II

Plant	Culture no.	Culture solution	Dry weight (in gm.)			Reducing sugars (in mg.)	
			Tops	Roots	Total	At termination of experiment	After 10 days' incubation
Corn	1	Pfeffer's solution alone	1.95	1.00	2.95	Trace	.....
	2		1.80	0.75	2.55	"	.....
	3		1.70	0.95	2.65	"	"
Pea	1		0.68	0.26	0.94	"	.....
	2		0.55	0.07	0.62	"	.....
	3		0.64	0.18	0.82	"	.....
Corn	1	Pfeffer's solution +0.35 per cent of soluble starch	0.7	0.4	1.10	3.4	4.0
	2		1.4	0.6	2.00	6.5	6.0
	3		1.5	0.7	2.20	3.7	4.0
	4		1.1	0.5	1.60	5.3	5.0
	5		1.4	0.6	2.00	4.4	4.0
Pea	1	Pfeffer's solution +0.35 per cent of soluble starch	0.5	0.2	0.70	3.7	4.0
	2		0.5	0.2	0.60	6.5	6.0
Control	1	No plant	.....	.....	.....	1.8	.....
	2		.....	.....	.....	3.7	.....

directly or indirectly, nor is there any appreciable secretion of amylase by the roots of these plants, at least under conditions such as were maintained for these experiments.

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